—m.p., 157.5–160° (corr.), not lowered by mixing with an authentic sample of ergosterol;[1] $[\alpha]^{25}_D$, −128.6; E_m, 11,160 (2,820 A.) (Fig. 1). The benzoate was prepared—m.p., 169.5–171° (corr.), not lowered when mixed with an authentic sample of ergosterol benzoate;[2] $[\alpha]^{25}_D$, −67.2.

Ergosterol has been detected in, and isolated in quantity from, the mycelium of *P. notatum* (X-1612) when the mold is grown in submerged culture.

References

1. BEALL, D., and GRANT, G. A. Unpublished data; cf., *Amer. chem. Soc. Abstr.* (Pittsburg Meeting), September 1943.
2. BIRKINSHAW, J. H., CALLOW, K. C., and FISCHMANN, C. F. *Biochem. J.*, 1931, **25**, 1977.
3. CAVALLITO, C. J. *Science*, 1944, **100**, 333.
4. OXFORD, A. E., and RAISTRICK, H. *Biochem. J.*, 1933. **27**, 1176.
5. PRUESS, L. M., PETERSON, W. H., STEENBOCK, H., and FRED, E. B. *J. biol. Chem.*, 1931, **90**, 369.
6. ZOOK, H. D., OAKWOOD, T. S., and WHITMORE, F. C. *Science*, 1944, **99**, 427.

The Effect on the Chick Embryo of the Simultaneous Inoculation of Stool, Streptomycin, and Penicillin

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Developing chick embryos were used in an attempt to isolate a virus from the stools of patients with epidemic diarrhea, nausea, and vomiting (*6*) (viral dysentery). To attempt to decrease the loss of virus due to filtration and yet eliminate bacterial growth, unfiltered stool suspensions were combined with penicillin, streptomycin, or combinations of both before inoculation of the embryo. Parker and Diefendorf (*5*) showed that the injection into chick embryos of 50–1,300 units of penicillin had no observable effect on the growth of the viruses of vaccinia, St. Louis encephalitis, or equine encephalomyelitis. Hirst (*3*) was able to increase the frequency of growth of influenza virus in chick embryos by the addition of penicillin to unfiltered throat washings. The protection of chick embryos from fowl typhoid by the use of streptomycin has been described by Jones, *et al.* (*4*). Florman and his co-workers (*2*) demonstrated the ability of influenza virus to grow in the presence of streptomycin in concentrations up to 12,000 units per egg. There was no evidence of any lethal effect on the developing embryo. Reimann, *et al.* (*7*) have observed that in the human colon 600 μg. (units) of streptomycin per gram of feces may in some instances be sufficient to kill all the colon bacilli.

[1] The physical constants of ergosterol (hydrate) are: m.p., 160–163°; $[\alpha]^{20}_D$, −128.7 (K. C. Callow. *Biochem. J.*, 1931, **25**, 79); Em, 11,500, (2,810 A.) (W. Huber, G. W. Ewing, and J. Kriger. *J. Amer. chem. Soc.*, 1945, **67**, 609).

[2] The physical constants of ergosterol benzoate are: m.p., 168–170°; $[\alpha]^{25}_D$, −70.5 (H. Wieland, and M. Assano. *Ann.*, 1929, **473**, 300).

Method

Thirteen- or 14-day-old developing chick embryos were inoculated, by the intra-amniotic method of Burnet (*1*), with 0.25–0.30 cc. of inoculum. The eggs were sealed with cellophane tape and incubated at 37° C. for approximately 40 hours. (This time was established during the early part of the study after noting the peak of the death rate of test and control eggs.)

Three different stool specimens were used: two which had been preserved on dry ice for 1–3 months, taken from persons suffering from epidemic diarrhea, nausea, and vomiting, and one fresh "normal" specimen. Ten-per cent suspensions of stool in broth were centrifuged at 1,500 r.p.m. for 10 minutes and the supernatant material collected for use as inoculum.

The solution of penicillin was prepared by dissolving the sodium salt in sterile physiologic saline to a final dilution of 25–200 units in 0.05 cc. Streptomycin hydrochloride was dissolved in sterile physiologic saline to make a solution containing 5,000 μg. (units)/0.05 cc.

TABLE 1

Group	Stool suspension (cc.)	Saline (cc.)	Penicillin (cc.)	Streptomycin (cc.)	No. eggs	Embryos		
						Living	Dead	Positive cultures
1	0.2	0.05	...	...	20	0	20	20
	...	0.25	...	...	20	14	6	3
2	0.2	...	0.05	...	20	2	18	20
	...	0.2	0.05	...	20	16	4	0
3	0.2	...	...	0.05	15	4	11	1
	...	0.2	...	0.05	15	7	8	0
4	0.2	...	0.05	0.05	20	10	10	1
	...	0.2	0.05	0.05	20	11	9	1

Penicillin: 0.05 cc. (25–200 units); streptomycin: 0.05 cc. (5,000 μg. or units); stool suspension: 0.2 cc. (10-per cent suspension).

Each inoculum containing stool suspension was allowed to stand at room temperature for 30–90 minutes before injection. A similar group of controls was performed, substituting sterile physiologic saline (0.2 cc.) for the stool suspension. The different types of inocula are outlined in Table 1.

After about 40 hours each egg was opened aseptically, the appearance of the embryo noted, and fluid removed for culture. This material from each egg was incubated in a tube of hormone broth for approximately 24 hours; then blood agar plates were inocu-

lated from each tube of broth. Special anaerobic culture methods were not attempted.

Results

The results are summarized in Table 1. Death of the embryos occurred in nearly all of the eggs which received stool suspension mixed with saline or with penicillin. These eggs usually were foul smelling, and the embryos often were in various stages of decomposition. Cultures from them were positive in every instance. In the control group inoculated with saline or penicillin, about two-thirds of the embryos survived and only 3 out of 40 had positive cultures, a contamination rate of about 7 per cent.

In contrast, half of the embryos survived in the eggs inoculated with stool suspension, streptomycin, and penicillin. Practically the same rate was obtained in the corresponding group of controls. About one-third survived when inoculated with stool suspension and streptomycin, a frequency below that of the control group. The number of positive cultures from all the eggs in these groups was about the same as the contamination rate in the controls.

Conclusions

By the described method, using streptomycin and penicillin in simultaneous inoculations of stool suspensions into chick embryos for the purpose of combating bacterial growth, this study suggests that the use of streptomycin and penicillin combined is of greatest value; streptomycin alone is probably inferior; and penicillin alone has no value.

References

1. Burnet, F. M. *Brit. J. exp. Path.*, 1940, **21**, 147.
2. Florman, A. L., Weiss, A. B., and Council, F. E. *Proc. Soc. exp. Biol. Med.*, 1946, **61**, 16.
3. Hirst, G. K. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 155.
4. Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A. *Science*, 1944, **100**, 103.
5. Parker, R. F., and Diefendorf, H. W. *Proc. Soc. exp. Biol. Med.*, 1944, **57**, 351.
6. Reimann, H. A., Hodges, J. H., and Price, A. H. *J. Amer. med. Ass.*, 1945, **127**, 1–5.
7. Reimann, H. A., Price, A. H., and Elias, W. F. *Arch. int. Med.*, 1945, **76**, 269–277.

Prothrombin and Fibrinolysin

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It has been stated a number of times that fibrinolysin, derived from plasma, can activate prothrombin to thrombin in the absence of calcium ion (*2, 3*). That conclusion has an important bearing on our understanding of the blood-clotting mechanism. Consequently, we were prompted to perform a series of experiments concerning the action of fibrinolysin on prothrombin. As a result of our work we believe that the fundamental conclusion is erroneous. When proper precautions are taken to *eliminate prothrombin and thrombin* from fibrinolysin preparations, the latter do not activate prothrombin, clot oxalated plasma, or clot purified fibrinogen solutions.

Quite unexpectedly, however, a new reaction has been discovered. Fibrinolysin *destroys* prothrombin. While the destruction proceeds it is possible to demonstrate, by commonly used analytical methods, that the purified prothrombin first becomes less easily activated with thromboplastin and later becomes completely refractive. We call this less reactive prothrombin *paraprothrombin,* to convey the idea that it is a modification of native plasma prothrombin. The reaction can be illustrated as follows:

$$\text{Prothrombin} \xrightarrow{\text{Fibrinolysin}} \text{Paraprothrombin} \longrightarrow \text{Inert protein}$$

Although the water-insoluble, saline-soluble, fibrinolysin preparation contains more than one protein, there is no reason to suspect that a factor other than the plasma-derived fibrinolytic principle is involved.

Fibrinolysin does *not* inactivate thrombin.

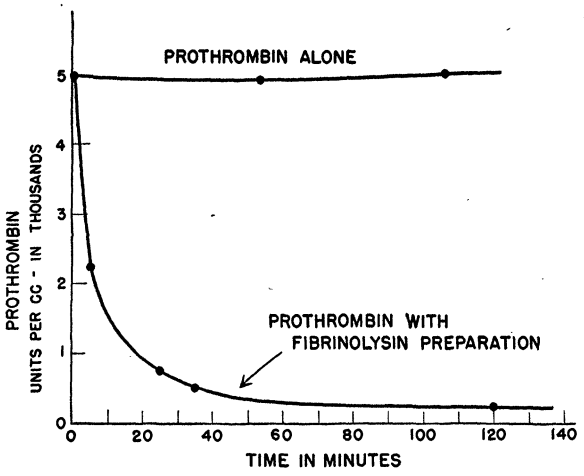

Fig. 1. The inactivation of purified prothrombin, at room temperature, with fibrinolysin.

Fig. 1 illustrates the rate of destruction of prothrombin by one of our potent lysin preparations. A 10,000-unit/ml. solution of purified prothrombin product No. 5 (*1*), dissolved in 0.9 per cent NaCl + 0.075 per cent $K_2C_2O_4 \cdot H_2O$, was mixed with an equal volume of fibrinolysin solution. The potency of the fibrinolysin solution was such that it would dissolve an equal volume of 0.3 per cent fibrin clot in less than 2 minutes. The disappearance of prothrombin was followed with the two-stage prothrombin titration technique (*4*).